ISSN (print) 0093-4666 © 2011. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/117.149

Volume 117, pp. 149–164 *July–September 2011*

A new volvate *Macrolepiota* (*Agaricomycetes*, *Agaricales*) from Italy, with observations on the *M. procera* complex

Alfredo Vizzini[1]*, Marco Contu[2], Stefano Ghignone[3], & Else Vellinga[4]

[1]*Dipartimento di Biologia Vegetale - Università degli Studi di Torino, Viale Mattioli 25, I-10125, Torino, Italy*
[2]*Via Marmilla, 12 (I Gioielli 2), I-07026 Olbia (OT), Italy*
[3] *Istituto per la Protezione delle Piante, CNR Sezione di Torino, Viale Mattioli 25, I-10125 Torino, Italy*
[4] *Department of Plant and Microbial Biology, 111 Koshland Hall #3102, UC Berkeley, Berkeley CA 94720-3102, U.S.A.*

* Correspondence to: *alfredo.vizzini@unito.it*

Abstract — A new *Macrolepiota* taxon from Italy, *M. rhodosperma* var. *velicopia*, is described and illustrated based on morphological and ITS rDNA data. It is characterized by a well-developed volva and abundant, evident velar remnants on the pileus, a stipe with a minutely squamulose covering, and very thick-walled elements in the pileipellis. A discussion on its taxonomic position within *Macrolepiota* and notes on closely related taxa are provided. DNA sequence analyses support the new taxon within the variability of *M. fuliginosa* sensu Vellinga, a non-volvate taxon that differs from Barla's original sense of *M. fuliginosa.* As Barla did not indicate a holotype in his protologue of *Lepiota procera* var. *fuliginosa* and there are no extant original herbarium specimens, Barla's Fig. 5/PL. 9 (from Les champignons des Alpes maritimes) is selected as a lectotype, and a recent collection from Liguria (Italy) is chosen as the epitype. A recent collection from Sardinia is chosen as epitype for *L. permixta*. Finally, *M. fuliginosa* and *M. permixta* are reduced in rank to forms of *M. procera*, based on their morphology.

Key words — *Agaricaceae,* biodiversity, taxonomy, *Volvolepiota*

Introduction

Macrolepiota Singer is a genus in the *Agaricaceae* characterized by basidiomes with a scaly pileus, a complex annulus, and a stipe with a smooth to granulose-squamulose covering and by spores with a distinct germ pore covered by a hyaline cap (Singer 1986). Species with a distinct volva were accommodated in the genus *Volvolepiota* Singer, proposed in 1959 to replace the illegitimate *Lepiotella* Rick (Rick 1938), a later homonym of *Lepiotella* (E.-J. Gilbert) Konrad.

The type of *Volvolepiota* is the Argentinean species *Lepiotella brunnea* Rick. Singer (1959) added a second species, *V. albida* Singer. In their monograph on *Volvolepiota* Heinemann & de Meijer (1996) argued that the presence of a well developed universal veil in this taxon was not sufficient to warrant a genus separate from *Macrolepiota* but did not propose new combinations in *Macrolepiota*.

Recent ITS-based molecular analyses by Vellinga (2003), Vellinga et al. (2003), and Ge et al. (2010) place *Volvolepiota* species nested in the *Macrolepiota* clade but in a basal position. Vellinga & Yang (2003), who considered *Volvolepiota* a synonym of *Macrolepiota*, proposed new *Macrolepiota* combinations and described *M. velosa,* a new volvate species from China. Ge et al. (2010) proposed *Macrolepiota* sect. *Volvatae* Z.W. Ge et al. to accommodate the volvate species. Four *Macrolepiota* taxa with a volva thus far known (from Australia, East Asia and South America; Vellinga & Yang 2003) all have a tropical distribution and relatively small spores.

Here we discuss our research on an Italian taxon with a copious universal veil and well-developed volva to determine its position within the genus. In the process we also studied *M. fuliginosa*, *M. permixta,* and the complex of *M. procera* (Scop.) Singer.

Materials & methods

Morphology

Macromorphological features were described from fresh specimens. Colour notations in the macroscopic descriptions are from Séguy (1936). Microscopical characters were made from dried material rehydrated in 5% KOH and stained in Congo red, Cresyl Blue, and Melzer's reagent. Spore measurements are based on means of 30 spores from two collections stained in Melzer's reagent. Basidia were measured where widest and from apex (sterigmata excluded) to basal septum. Author citations follow Index Fungorum (http://www.indexfungorum.org/authorsoffungalnames.htm). Herbarium abbreviations follow Thiers (2011). All examined material is housed at TO (Herbarium generale del Dipartimento di Biologia Vegetale, Università degli Studi di Torino, Italy). The new Latin description is deposited in MycoBank (http://www.mycobank.org/).

The following abbreviations are used: Q = quotient of length and width of spores in side view; Q_m = average quotient; L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; (Se) = Séguy (1936).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of herbarium specimens (Table 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 (White et al. 1990; Gardes & Bruns 1993) were used to amplify the ITS1+5.8S+ITS2 rDNA region between the small (18S gene) and large (28S gene) subunits. Amplifications were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixtures containing 5 ng

Table 1. *Macrolepiota* samples newly sequenced in this study.

Sample no.	Species	Country — Location	Collector Sampling date [herb. number]	Habitat	ITS acc. no.
1	*M. fuliginosa*	Italy — Sardinia, Olbia-Tempio, Rena Majore	M. Contu 15/XI/2009 [TO HG2006]	Coastal pinewood	HM246501
2	*M. fuliginosa*	Italy — Liguria, Savona, Borgio Verezzi	A. Vizzini 09/X/1998 [TO AFM11]	*Quercus ilex* litter	HM246502
3	*M. permixta*	Italy — Sardinia, M.S. Vittoria Esterzili	M. Casula 07/XII/2007 [TO AFM12]	*Pinus* sp. litter	HM246503
4	*M. rhodosperma* var. *velicopia*	Italy — Piedmont, Turin, Giaveno, Colletto del Forno	A. Vizzini 07/X/2008 [TO HG2003]	*Fagus sylvatica* litter	HM246505
5	*M. rhodosperma* var. *velicopia*	Italy — Piedmont, Turin, Venaria Reale, Parco Regionale La Mandria	L. Latino 15/X/2008 [TO HG2004]	*Quercus robur* & *Q. petraea* litter	HM246506
6	*M. rhodosperma*	Italy — Piedmont, Turin, Venaria Reale, Parco Regionale La Mandria	A. Vizzini 20/X/2008 [TO HG2005]	*Quercus rubra* & *Corylus avellana* litter	HM246507
7	*M. excoriata* (Schaeff.) Wasser	Italy — Piedmont, Turin, Colle del Frais	A. Vizzini 16/VIII/2008 [TO AFM13]	Pasture	HM246504

DNA, 1x PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 μM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.5 unit of Taq polymerase (Promega). PCR run times were 3 min at 95°C (1 cycle), 30 s at 94°C, 45 s at 50°C, 2 min at 72 °C (35 cycles), and 10 min at 72°C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy). Sequences were assembled and edited with the phred/phrap/consed software suite (Gordon et al. 1998). The sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Sequence alignment and phylogenetic analysis

A representative set of the ITS1-5.8S-ITS2 regions of Eurasian and Australian *Macrolepiota* taxa and two *Leucoagaricus* species was retrieved from Genbank. The sequence alignment was performed by MAFFT version 6 (Katoh et al. 2002; Katoh & Toh 2008) using default settings. After minimal visual fine-tuning of the alignment, a final sequence set with a total 775 database length comprised 65 collections from different studies.

A Bayesian Metropolis-coupled Markov chain Monte Carlo (BI) analysis was conducted with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The model of nucleotide substitution for the BI analysis was selected using ModelTest v. 3.7 (Posada & Crandall 1998). The transversional substitution model with gamma correction (TVM + G)

was chosen as the best-fit model based on hierarchical likelihood tests, following the Akaike Information Criterion (AIC). The general structure (lset) of the substitution model was defined using nst=6 rates=gamma ngammacat=4; TVM + G model was gained using the following parameters to define the priors: the four stationary frequencies of the nucleotides (statefreqpr=fixed(0.2310, 0.2236, 0.2349, 0.3105)), the six different nucleotide substitution rates (revmatpr=fixed(1.7916, 5.3304, 2.2627, 0.7366, 5.3304, 1.00)), the proportion of invariable sites (pinvarpr=fixed(0.00)), and the shape (shapepr=fixed(0.3944)). Markov chains were run for 10^6 generations, with the sampling frequency set every 100th generation; all other parameters were used at the default settings. The outgroup *Leucoagaricus nympharum* was selected to root the tree. A burnin value set to 25% of sampled trees was used to calculate the parameters and the consensus tree.

The data set was also analyzed by a maximum likelihood (ML) method using RAxML version 7.2.3 (Stamatakis et al. 2008). One hundred rapid ML bootstraps were performed, using default settings. The phylogenetic trees were visualized and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree). The alignment was deposited in TreeBase (www.treebase.org) under study accession no. S10573.

Results & discussion

Phylogeny based on the ITS dataset

The ITS amplification products of the analysed samples ranged from 344 to 680 bp. A total of 65 sequences were used for the ITS phylogeny, including the seven newly sequenced collections (Tab. 1) and all others retrieved from GenBank (accession numbers are given in Fig. 3). The multiple sequence alignment was 775 characters long in total.

The phylogenetic trees resulting from the two types of analysis do not differ in topology. The Bayesian inference consensus tree (Fig. 3) indicates that our volvate taxon clusters together with *M. rhodosperma* (P.D. Orton) Migl. (AY243596) and with isolates formerly identified as *M. fuliginosa*, and one accession under the name *M. konradii* (P.D. Orton) M.M. Moser. The two sequences from our volvate *Macrolepiota* differ among themselves and from *M. fuliginosa* and *M. rhodosperma* sequences in only1–2 bases in three different combinations. This clade, supported by a 1.0 posterior probability value and 98% ML bootstrap value, is sister to all other species in sect. *Macrolepiota*, except for *M. clelandii* Grgur.

ITS sequences of taxa identified as *M. permixta* and *M. fuliginosa* do not differ from those of *M. procera*.

The group of *M. mastoidea* (Fr.) Singer, although homogeneous in sequences, represents basidiomes with quite different morphologies, and these have been accessed under a range of species names; an in-depth multigene analysis of this complex is needed.

All three sections, sect. *Macrolepiota*, sect. *Macrosporae* (Singer) Bon, and sect. *Volvatae* are recovered in the phylogenies, and well supported.

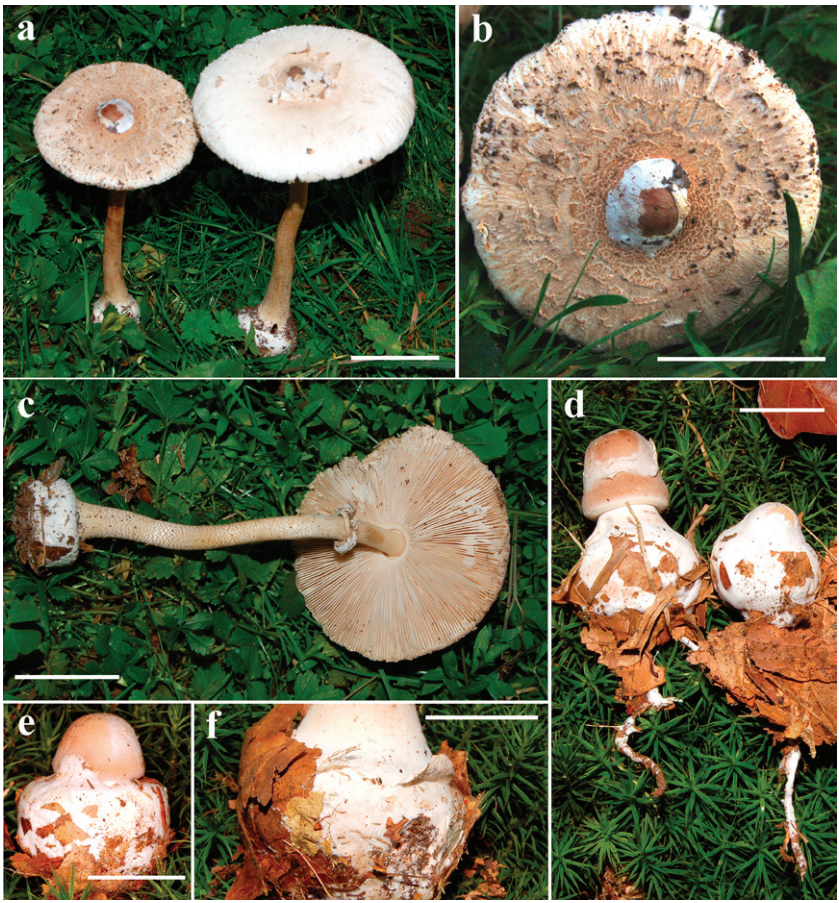

Figure 1. *Macrolepiota rhodosperma* var. *velicopia*. Macroscopic features (a–c from the holotype; d–f from TO HG2003). **a**. Mature basidiomes. **b**. Pileus surface with evident velar patches. **c**. Flattened bulb and ornamented stipe. **d**. Young basidiomes covered with universal veil patches and with well developed basal rhizomorphs. **e–f**. Submarginate bulb with free velar limbs (volva). Scale bars: a–c = 5 cm; d–f = 2 cm.

Taxonomy

Macrolepiota rhodosperma var. ***velicopia*** Vizzini & Contu, **var. nov.** Figs. 1–2

MycoBank MB518426

A typo differt velo albo membranoso ad instar Amanitarum in pileo et ad basim stipitis manifesto, hyphis pilei cutis conspicue crassotunicatis, sporis minoribus, fibulisque haud fraequentibus.

Holotypus — *Italia, in regione Piemonte dicta, ad locum dictum Parco Regionale La Mandria (Venaria Reale, Torino), 15.X.2008, leg. L. Latino (TO HG2004).*

Etymology — the varietal epithet, derived from the Latin words *velum, veli* = veil, velum; and *copiă, copiae* = abundance, emphasizes the copious veil.

Pileus up to 15 cm wide when expanded, at first parabolic then plano-convex, with a low, wide, central umbo, dry, when young entirely covered with a granulose velvety covering, later breaking into a central calotte, which may be round to irregularly star-shaped, felted-subsquamulose, up to ½ of radius wide, cream hazel (Se 134, 695) to light chestnut brown (Se 111, 131), surrounded by 2–10 mm wide and easily removable lighter patches with curled edges, on a radially coarsely floccose-fibrillose covering made up of chamois beige to pale brown fibrillose strands on a white background; with white to dirty white membranous velar remnants as patches on the surface; margin slightly to distinctly fringed and exceeding lamellae. Lamellae, L = 80–140, l = 0–3, free and remote from the stipe, with an evident collarium, moderately crowded, up to 9 mm broad, at first whitish then with cream to pinkish tinges, with a fimbriate-floccose concolorous edge. Stipe protruding into the pileus, (5–) 9–18(–22) × (0.5–)0.8–1.5(–1.8) cm, cylindrical to slightly tapering towards the apex, hollow, bulbous at the base, with an oblong to flattened submarginate bulb, (2–)2.5–3.2(–3.5) × 3–4 cm, often with long white rhizomorphs; with adnate minute cream-hazel zigzagging bands over the whole length, on a white background; bulb white tomentose with evident and membranous white velar remnants on the margin. Annulus movable with age, complex, thickened with a relatively broad edge, upperside creamy and fringed and underside hazel. Context white, unchanging, both on handling and in ammonia vapour, up to 13 mm thick in the pileus; smell indistinct, and taste indistinct to mild. Spore-print whitish-cream.

Spores (13–)13.5–15(–16.5) × 9–10.5(–11.2) µm, on average 14.55 × 9.93 µm, Q = 1.4–1.6, Q_m = 1.46, ellipsoid to oblong, ovoid, hyaline, smooth, thick-walled, with walls easily swelling in 5% KOH, with an apical and central germ-pore (1–1.5 µm wide) and hyaline cap (callus) on it, with a small apiculus, dextrinoid, cyanophilous, congophilous, with inner wall metachromatic in Cresyl Blue (Fig. 2a); basidia 25–42 × 9–16 µm, 4-spored, some 1–2-spored, broadly clavate (Fig. 2b); subhymenium well-differentiated, cellular; hymenophoral trama subregular to slightly interwoven; pleurocystidia absent; lamellar edge sterile, consisting of tightly packed cheilocystidia; cheilocystidia 25–50(–60) × 10–15 µm, abundant, very variable in shape and size, narrowly clavate to sub-fusiform, seldom also lageniform, utriform or cylindrical, sometimes with a small capitulum, rarely with an apical excrescence, often catenulate (pluricellular) septate in lower part, colourless and thin-walled, non-encrusted (Fig. 2c); pileus covering (in calotte) a trichoderm of confusedly erect, (4.5–)6–12 µm wide hyphae ending in a subfusiform (gradually tapering toward the apex) terminal element with several secondary septa (pseudosepta),

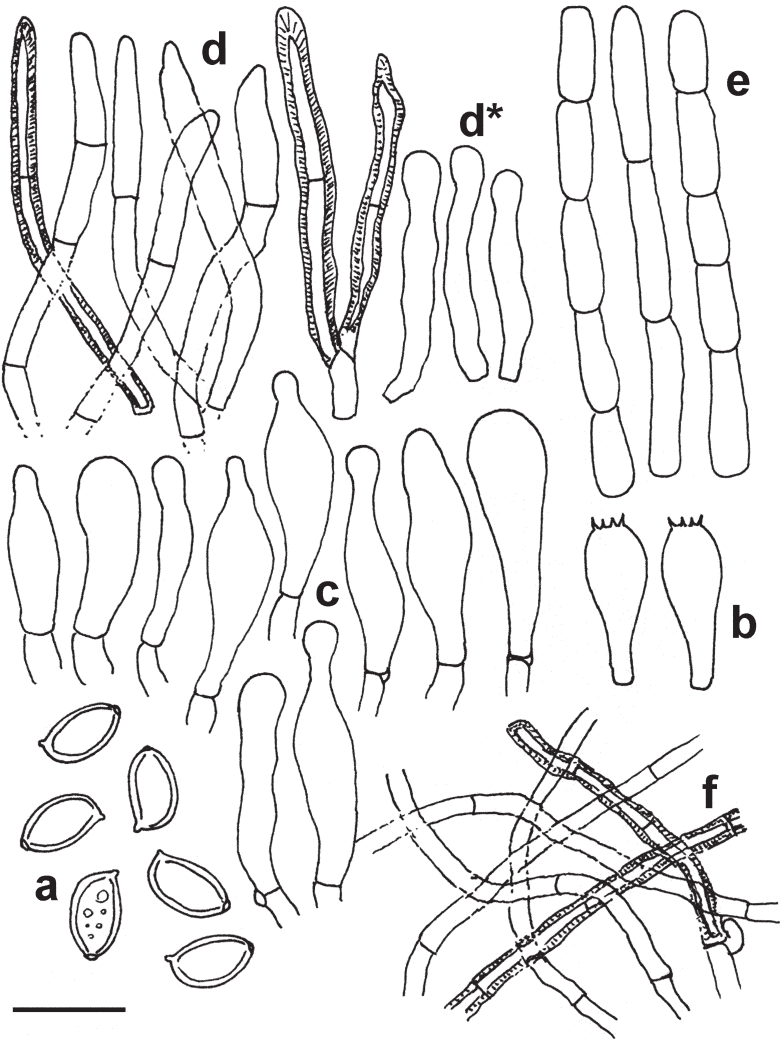

Figure 2. *Macrolepiota rhodosperma* var. *velicopia*. Microscopic features (from the holotype). **a**. Spores. **b**. Basidia. **c**. Cheilocystidia. **d**. Pileus covering. **d***. Pileus covering underlying elements. **e**. Annulus elements. **f**. Velar elements. Scale bar: a–c = 20 μm; d–f = 30 μm.

some very thick-walled (1.5–2 µm thick), up to 250 µm long, with a narrow lumen, and then resembling the setiform terminal elements in the pileipellis of *Crinipellis* species, other thin-walled and shorter (Fig. 2d); at the base of the trichodermial elements there is a loose discontinuous layer made up of short cylindrical to subcapitulate elements (Fig. 2d*). Pigmentation in the form of brownish pigment, parietal and cytoplasmatic in the thick-walled hyphae, parietal and minutely encrusting in the thin-walled hyphae, especially towards their base. Annulus consisting of tightly packed, catenulate, articulate, and short cylindroid hyphae, 4.5–9 µm wide, with rare subglobular elements (Fig. 2e). Velar patches made up of elongate, cylindrical hyphae, thin-walled, septate, 3–11 µm wide, sometimes branched or nodulose, with scattered pseudoclamp connections (proliferating clamps), intermixed with thick-walled hyphae (cell wall up to 1.5 µm thick), up to 10.5 µm wide, with internal lumen, looking like the crinipelloid elements present in the pileus covering, probably derived from the underlying pileipellis (Fig. 2f). Clamp connections rare, small, observed at the base of the cheilocystidia; thromboplerous hyphae absent.

Habitat and distribution solitary, terrestrial, on litter of broad-leaved trees, especially *Fagaceae* (*Fagus* and *Quercus*). So far known only from Italy.

Additional material studied: ***Macrolepiota rhodosperma* var. *velicopia*** **–ITALY**, **Piedmont**, Turin, Giaveno, loc. Colletto del Forno, 7 Oct 2008, litter of *Fagus sylvatica* L., 1000 m asl, leg. A. Vizzini (TO HG2003); ***M. rhodosperma* var. *rhodosperma*** **– ITALY**, **Piedmont**, Turin, Venaria Reale, Parco Regionale La Mandria, 20 Oct 2008, litter of *Quercus rubra* L. and *Corylus avellana* L., 230 m asl, leg. A. Vizzini (TO HG2005).

Macrolepiota rhodosperma var. *velicopia* was collected from two different localities in northern Italy. A third collection turned out to be infected by a hyphomycete species that covered the pileus and base of the stipe. Such infections are very rare in the *Agaricaceae*.

Morphologically, *M. rhodosperma* var. *velicopia* is very different from the other volvate *Macrolepiota* species because of such peculiar features as an obviously decorated stipe, spores easily reaching 14–15 µm in length, not strictly cylindrical cheilocystidia, and long very thick-walled elements in the pileus covering. This taxon is the first volvate *Macrolepiota* reported in Europe.

The South American *M. pulchella* de Meijer & Vellinga [≡ *Volvolepiota brunnea* (Rick) Singer] and *M. brunnescens* Vellinga [≡ *V. albida* Singer] differ in smaller spores that rarely reach 11 µm in length, a stipe lacking any decoration, and a less complex annulus; furthermore *M. brunnescens* has a white context turning pink-brown after handling (Heinemann & de Meijer 1996). *Macrolepiota eucharis* Vellinga & Halling from northeast Australia has a smaller pileus (≤ 6 cm broad when fully expanded), a pileus decorated with tiny black scales, a simple ascending annulus that is white on both sides, a uniformly fibrillose dark brown stipe, narrower spores, narrower cylindrical cheilocystidia, and no clamp connections (Vellinga 2003).

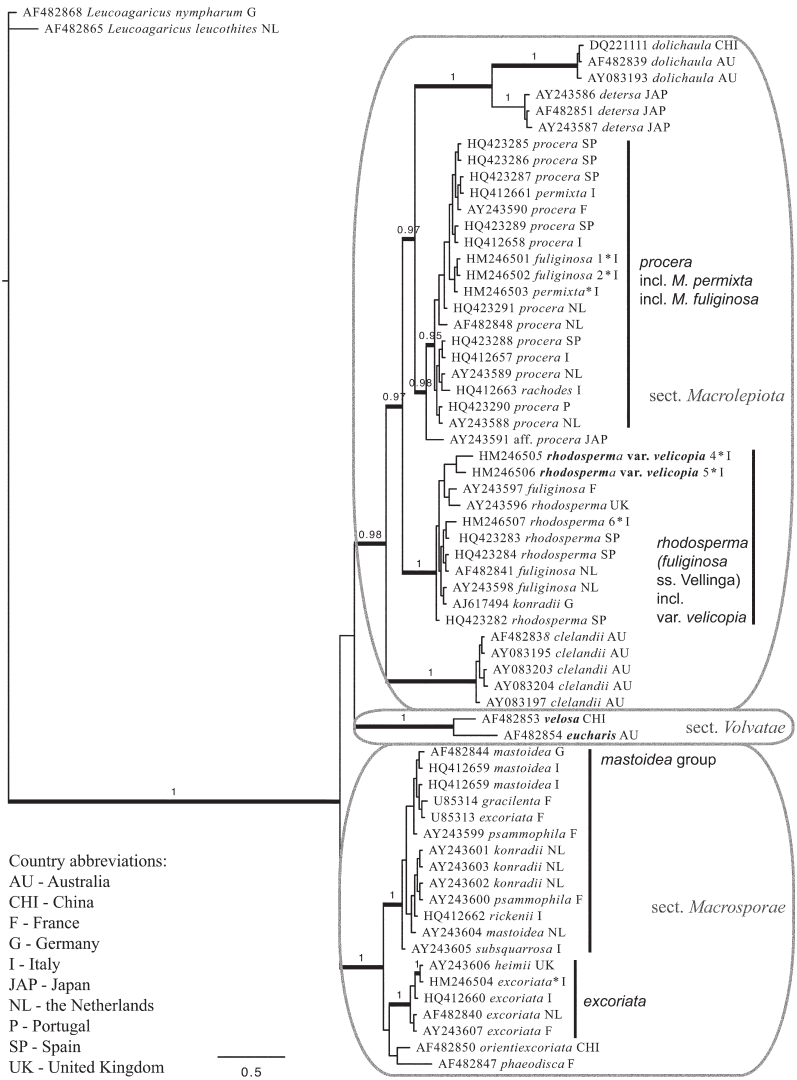

Figure 3. ITS-rDNA phylogeny inferred by Bayesian analysis. Bayesian posterior probability values greater than 95%, derived from 10.000 Markov chain Monte Carlo sampled trees, are given above branches; thick branches indicate ≥75% ML bootstrap values from the RAxML analysis. The tree is rooted using *Leucoagaricus nympharum* as outgroup. Volvate *Macrolepiota* taxa are in bold. * indicates samples sequenced in this work and reported in Table 1. Names appear as in GenBank except for two recently described species from China (Ge et al. 2010). The bar indicates number of substitutions per site.

Macrolepiota velosa Vellinga & Zhu L. Yang, from south-western China and northern Thailand, is characterized by a 7–9 cm wide pileus with dark brown to purple scales, a finely fibrillose or squamulose stipe, a less complex annulus, very small (8–10(–11) × 6–7 µm) spores, longer and strictly cylindrical cheilocystidia measuring 44–68 × 4.5–7.5 µm, and the absence of clamp connections (Vellinga & Yang 2003; Ge et al. 2010).

Among the poorly studied, insufficiently known taxa, "*M. nordica* var. *subvelata*" Bon ad int. (Bon 1993) comes quite close to our taxon but differs in a less decorated stipe and a different pileipellis structure comprising articulated hyphae.

Our volvate taxon clusters with two *Macrolepiota* taxa that also exhibit thick-walled elements in the pileus covering: *M. fuliginosa* sensu Vellinga (Vellinga 2001, Vellinga et al. 2003) and *M. rhodosperma* lacking such a volva and velar remnants (Fig. 3); *M. rhodosperma* is considered a synonym of *M. fuliginosa* sensu Vellinga (Vellinga 2001, Vellinga et al. 2003).

Our phylogenetic analyses (Fig. 3) clearly demonstrate that *M. rhodosperma* var. *velicopia* is not closely related to the other known volvate taxa (*M. eucharis, M. velosa*), which implies that velar structure acquisition or loss is a homoplastic character that developed independently during the evolution of *Macrolepiota*, which makes it unsuitable for a natural classification of these fungi and further emphasizes the artificial nature of *Volvolepiota*.

Macrolepiota procera* f. *fuliginosa (Barla) Vizzini & Contu, **comb. nov.**

MycoBank MB518708

≡ *Lepiota procera* var. *fuliginosa* Barla, Champ. Alp. Marit.: 21, pl. 9 fig. 5, 1888.

≡ *Leucocoprinus fuliginosus* (Barla) Locq., Bull. Soc. Linn. Lyon 14: 92, 1945.

≡ *Macrolepiota fuliginosa* (Barla) Bon, Docum. Mycol. 7(27–28): 20, 1977.

≡ *Macrolepiota procera* var. *fuliginosa* (Barla) Bellù & Lanzoni, Beitr. Kenntn. Pilze Mitteleurop. 3: 190, 1987.

Type: **France**, **Alpes-Maritimes**, illustration in Les champignons des Alpes maritimes (Barla 1888: pl. 9 fig. 5, iconotype, **lectotype designated here**). **Italy**, **Liguria**, Savona, Borgio Verezzi, in *Quercus ilex* litter, 09/10/1998, leg. et det. A. Vizzini (Fig. 5a; TO AFM11, **epitype designated here**).

Lepiota procera var. *fuliginosa*, described from Mediterranean France (Barla 1888), has been differently interpreted by subsequent authors due to the lack of original material (Trimbach 1996). According to mycologists with a vast Mediterranean experience (Bellù & Lanzoni 1987, Candusso & Lanzoni 1990, Bon 1993, Migliozzi 1995), this taxon comes very close to *Macrolepiota procera* from which it differs mainly in the darker tinged pileus and stipe, the hardly disrupted stipe covering, and the browning context. These authors describe the pileipellis of *M. fuliginosa* as a trichoderm made up of thin-walled to only slightly thick-walled hyphae due to the presence of a parietal pigment.

FIGURE 4. *Lepiota procera* var. *fuliginosa* (lectotype, Barla 1888: pl. 9 fig. 5).

Other authors, including Vellinga (2001), Lange & Vellinga (2004), and Lange (2008), described under the name "*Macrolepiota fuliginosa*" a different agaric, characterised in part by a pileus with grey-brown, loose and easily removable scales and a pileipellis comprising very thick-walled elements with brown intracellular and encrusting pigments. This latter taxon is widespread and known from Denmark southwards into Spain (see FIG. 3).

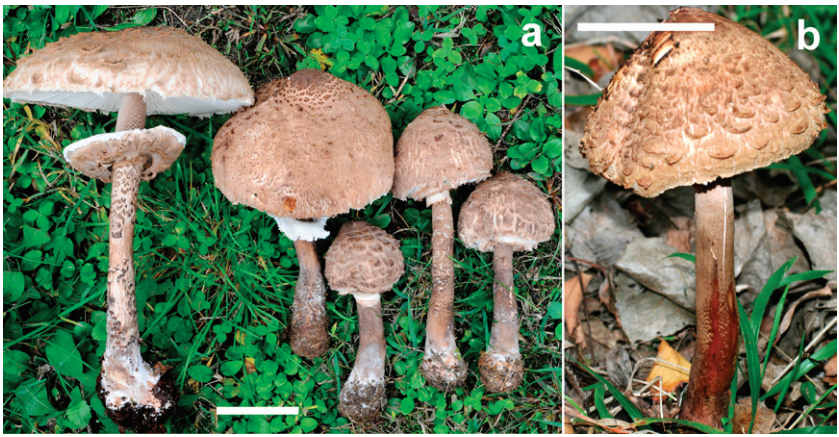

Figure 5. **a**. *Macrolepiota procera* f. *fuliginosa* (epitype, TO AFM11). **b**. *M. procera* f. *permixta* (epitype, TO AFM12). Bars = 5 cm.

Our ITS sequence analysis, which includes two Mediterranean collections (from Sardinia and Liguria, Italy; Tab. 1), shows that *M. fuliginosa* sensu Vellinga differs from *M. fuliginosa* sensu auct. pl. (Fig. 3). We regard *Lepiota procera* var. *fuliginosa* as a predominantly Mediterranean, xerophilous taxon that is correctly delimited only when based upon Mediterranean records. Consequently, we designate the original Figure 5/Pl. 9 included by Barla (1888) in the protologue (Fig. 4) as its lectotype (iconotype) and select a recent collection from Liguria – where the Mediterranean taxon is rather widespread – as epitype. The basidiomata of the epitypical collection here selected are identical, in gross characters, with those depicted by Barla (1888) and exhibit microscopic features perfectly fitting the descriptions reported by many authors (Bellù & Lanzoni 1987, Candusso & Lanzoni 1990, Bon 1993, Migliozzi 1995) in the region. It is also clear from Fig. 3 that the ITS sequences do not separate this taxon from *M. procera*. However, morphological comparisons do support its recognition as a form of *M. procera* as noted by Bellù & Lanzoni (1987), Candusso & Lanzoni (1990), and Migliozzi (1995). Therefore we introduce the necessary new combination.

The name *Macrolepiota rhodosperma* is available for *M. fuliginosa* sensu Vellinga. Notes on the synonymy of the two are given by Vellinga (2001) and Vellinga et al. (2003). A good picture is provided by Breitenbach & Kränzlin (1995, fig. 250 as *M. konradii)*. *Macrolepiota rhodosperma* var. *rhodosperma,* which lacks the copious velar remnants of var. *velicopia*, also has longer spores and a context that changes brown-red.

Figure 6. *Lepiota permixta* (Barla 1888: pl. 11 figs 1–4).

Macrolepiota procera f. ***permixta*** (Barla) Vizzini & Contu, **comb. nov.**

MycoBank MB518709

≡ *Lepiota permixta* Barla, Bull. Soc. Myc. Fr. 2: 114, 1886.

≡ *Leucocoprinus permixtus* (Barla) Locq., Bull. Soc. Linn. Lyon 14: 92, 1945.

≡ *Macrolepiota permixta* (Barla) Pacioni, Micol. Ital. 8(3):13, 1979.

≡ *Macrolepiota procera* var. *permixta* (Barla) Quadr., in Quadraccia & Lunghini, Quad. Acc. Naz. Lincei 264: 110, 1990.

Type: **FRANCE**, **Alpes-Maritimes**, Bendejeun, 7/12/1893, Barla Myc.283 (NICE, **lectotype**, designated in Trimbach 1996: 226). **ITALY**, **Sardinia**, M.S. Vittoria Esterzili, in *Pinus* litter, 07/12/2007, leg. M. Casula, det. M. Contu (Fig. 5b; TO AFM12, **epitype designated here**).

Barla (1886) described *Lepiota permixta* as a large long-stemmed species characterised by a pileus that is "10–15" cm wide, "brun-cannelle-fauve au centre," "brunâtre" towards the margin, "à écailles plus ou moins apprimées, fibrilleux, blanchâtre et plus ou moins écorché vers la marge", lamellae that are yellowish-white or "charné-clair", a very slender white stipe with "petites écailles appliquées, irrégulières, brunâtres, écorché vers la marge", a membranous "fauve-brunâtre" annulus, and a reddening context. He added that the described basidiomes were collected in a submontane region and that the species was rare. In the short notes accompanying the French description, Barla stated that *Lepiota permixta* combined features from three species: the slender habit of *L. procera*, the "écorché à la marge" pileus of *L. excoriata*, and the reddening context of *L. rachodes.* The colour plate later published by Barla (1888) displays basidiomes exhibiting the very same features as described in the protologue and with an annulus that apparently differs from that typically described for *Macrolepiota procera* complex (Fig. 6). Most mycologists, especially those in the Mediterranean region, have treated *Lepiota permixta* as a species separate from the *M. procera* complex, as supported by the clearly reddening context (e.g., Pacioni 1979 with a very good description of Sardinian material, Bon 1993, Bellù & Lanzoni 1987, Pázmány, 1985). Others (Candusso & Lanzoni 1990, Migliozzi 1995, 1997, Quadraccia & Lunghini 1990) consider *M. permixta* a variety of *M. procera,* assuming that the presence or absence of reddening is not sufficient to distinguish the two taxa at species level. Pázmány (1988) showed the great macromorphological variability of *M. procera* by describing eight infraspecific taxa. Since our selected *L. permixta* epitype and other collections identified as *M. permixta* share nearly or completely identical ITS sequences with *M. procera* and *M. fuliginosa*, we reduce *M. permixta* to a morphological form of *M. procera* and propose a new taxonomic combination.

Acknowledgements

We would like to thank Prof. E. Grilli (Popoli, Italy) for improving the English text and G. Eyssartier (Paris, France) and Prof. Fernando Esteve (Alcalá de Henares, Spain) for their pre-submission reviews. Our sincere thanks also to Dr. P.A. Moreau (Lille, France) for providing helpful suggestions. SG benefited from a Compagnia di San Paolo grant. Dr. Todd Osmundson and Lavinia Latino kindly provided us with pre-submission *Macrolepiota* sequences and with *M. rhodosperma* var. *velicopia* pictures, respectively, and ECV acknowledges funding from NSF grant DEB 0618293.

Literature cited

Barla JB. 1886. Liste des champignons observés dans le Département des Alpes Maritimes. Bull. Soc. Mycol. France 2(3): 112–119.

Barla JB. 1888. Flore mycologique illustrée. Les champignons des Alpes maritimes avec l'indication de leur propriétés utiles ou nuisibles. A. Gilletta, Nice. 80 p., 69 pl.

Bellù F, Lanzoni G. 1987. Betrachtungen über die Gattung *Macrolepiota* Singer in Europa. Beiträge zur Kenntnis der Pilze Mitteleuropas 3: 189–204.

Bon M. 1993. Flore mycologique d'Europe 3. Les Lépiotes. Docum. Mycol. Mém. Hors-Série 3: 1–153.

Breitenbach J, Kränzlin F. 1995. Champignons de Suisse, Tome 4: Champignons à lames, 2ème partie: *Entolomataceae*, *Pluteaceae*, *Amanitaceae*, *Agaricaceae*, *Coprinaceae, Bolbitiaceae, Strophariaceae*. Ed. Mykologia, Luzern.

Candusso M, Lanzoni G. 1990. *Lepiota* s.l. Fungi Europaei 4. Giovanna Biella, Saronno.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2(2): 113–118. http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x

Ge ZW, Yang ZL, Vellinga EC. 2010. The genus *Macrolepiota* (Agaricaceae, Basidiomycota) in China. Fungal Diversity 45: 81–98. http://dx.doi.org/10.1007/s13225-010-0062-0

Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. Genome Res. 8: 195–202.

Heinemann P, Meijer AAR de 1996. The status of *Volvolepiota* Sing. Bull. Jard. Bot. Natl. Belg. 65: 405–412. http://dx.doi.org/10.2307/3668462

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30: 3059–3066. http://dx.doi.org/10.1093/nar/gkf436

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298. http://dx.doi.org/10.1093/bib/bbn013

Lange C. 2008. *Macrolepiota* Singer. 554–557, in: H. Knudsen, J Vesterholt (eds). Funga Nordica – Agaricoid, boletoid and cyphelloid genera. Nordsvamp, Copenhagen, Denmark.

Lange C, Vellinga EC. 2004. Rabarber-parasolhat holder flyttedag – molekylær-genetiske studier omkring slægten *Macrolepiota*. Svampe 50: 23–42.

Migliozzi V. 1995. Un genere alla volta. Introduzione allo studio del genere *Macrolepiota* Singer. Boll. Gr. Micol. G. Bresadola 38(5–6): 131–148.

Migliozzi V. 1997. Note introduttive allo studio delle *Lepiotaceae*. Pagine di Micologia 8: 1–64.

Pacioni G. 1979. Flora micologica della Sardegna: un contributo. Micol. Ital. 8(3): 11–16.

Pázmány D. 1985. A *Macrolepiota* nemzetség euròpai fajainak hatàrozòkulcsa. Mikol. Közlem. 3: 115–136.

Pázmány D. 1989 ("1988"). Über den Formenkreis der *Macrolepiota procera*. Art. Not. Bot. Horti Agrobot. Cluj-Napoca 18–19: 5–22.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9): 817–818. http://dx.doi.org/10.1093/bioinformatics/14.9.817

Quadraccia L, Lunghini D. 1990. Contributo alla conoscenza dei macromiceti della Tenuta Presidenziale di Castelporziano (micoflora del Lazio II). Quad. Acc. Naz. Lincei 264: 49–120.

Rick J. 1938. Agarici Riograndenses II. Lilloa 2: 251–316.

Séguy E. 1936. Code universel des couleurs. Paul Chevalier, Paris.

Singer R. 1959. Dos géneros de hongos nuevos para Argentina. Bol. Soc. Argent. Bot. 8: 9–13.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 75: 758–771. http://dx.doi.org/10.1080/10635150802429642

Thiers B. 2011. (continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Trimbach J. 1996. Barla et la Mycologie. 179–256, in: G Thomel (ed.). Jean-Baptiste Barla 1817–1896. Volume publié à l'occasion du centenaire de sa mort. Annales du Museum d'Histoire Naturelle de Nice Tome 11.

Vellinga EC. 2001. *Macrolepiota*. 64–73, in: ME Noordeloos et al. (eds). Flora Agaricina Neerlandica 5. A.A. Balkema Publishers, Lisse, Abingdon, Exton (PA), Tokyo.

Vellinga EC. 2003. *Chlorophyllum* and *Macrolepiota* (*Agaricaceae*) in Australia. Aust. Syst. Bot. 16: 361–370. http://dx.doi.org/10.1071/SB02013

Vellinga EC, Yang ZL. 2003. *Volvolepiota* and *Macrolepiota* – *Macrolepiota velosa*, a new species from China. Mycotaxon 85: 183–186.

Vellinga EC, de Kok RPJ, Bruns TD. 2003. Phylogeny and taxonomy of *Macrolepiota* (*Agaricaceae*). Mycologia 95: 442–456. http://dx.doi.org/10.2307/3761886

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols. Academic Press, London.